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SHORT COMMUNICATION

The production of sporostatic factors in soil

Summary

Sixteen species of fungi were isolated from a park-land soil. When inoculated back into a sterilized sample of this soil three sporostatic factors, acetaldehyde, n–propanol and nonanoic acid were found to accumulate and the soil became inhibitory to the germination of test spores. The level of inhibition recorded could be explained wholly in terms of the prevailing levels of the identified sporostatic factors.

Introduction

An inhibitor of fungal spore germination has been widely reported in soil, and has been attributed variously to lack of nutrients and to the presence of germination inhibitors. Lingappa and Lockwood [7] are amongst those who prefer the nutrient deficiency hypothesis and Lockwood [9] has pointed out that the interpretation of indirect methods of assay for mycostasis in soil is frequently complicated since the test spores are removed from contact with the soil, and the use of carrier media together with nutrients leached from the test spores can stimulate the local activity of antibiotic producers (The term antibiotic is used here as defined by Waksman [21]). Thus these indirect methods do not discriminate between the pre-existence of an inhibitor in soil and its production during the assay period. Whilst these criticisms are valid, many authors have demonstrated inhibitory activity in extracts of soil [1 2 3 5 8 10 17 19 22]. Moreover, it can be shown that soil contains sufficient nutrients to support the germination of a wide range of fungal spores [2 6 7 13]. It seems likely, therefore, that major importance should be ascribed to antibiotic substances as causal agents in the inhibition of fungal spore germination in soil.

Park [12] has reviewed evidence that the inhibitor in soil is of microbial origin. Unfortunately this evidence is circumstantial and the present work outlines an attempt to obtain direct evidence for the production of sporostatic factors [15] in soil. Previous work has emphasized the similarity between the inhibition of spore germination in soils and sporostasis in laboratory cultures of fungi [4 14 16], so it seemed logical to look in soil for those factors contributing to sporostasis in laboratory cultures.

Materials and methods

Three 1-kg samples of soil were obtained from an open-canopy wooded area containing mature trees of *Acer Pseudoplatanus* L., *Fagus sylvatica* L., *Quercus Robar* L. and *Tilia vulgaris* Hayne, in the Barnett Demesne, Belfast. Fungi were isolated from the soil by Warcup plates [20] and soil-dilution (1:5000 w/v) platings using rose-bengal agar.[18] The fungi were subsequently identified in pure culture on either Oxoid Czapek Dox agar or Oxoid potato dextrose agar.

The soil samples were sterilized by autoclaving for 20 min at 15 psi. One of the soil samples was placed over a glass sinter and leached by passing 25 l of sterile distilled water through it for a period of 8 h. After leaching, the sample was allowed to air dry to 1 kg at room temperature, and the crumb structure of the remaining two soil samples was adjusted to match that of the leached soil as closely as possible.

The fungi which had been isolated from the soil were then inoculated back into the leached and one of the unleached samples. To facilitate this, the fungi were grown on washed and autoclaved 'Cellophane' placed over Czapek Dox agar, then single colonies (5 cm diameter) of each of the isolates were peeled off the 'Cellophane' film and mixed into the soil sample. The two inoculated soils, together with the sterile control sample were incubated at $20 \pm 2°C$ for 3 weeks. After this period the level of inhibition of spore germination in each of the soil samples was estimated by placing on their surface strips of washed and autoclaved 'Cellophane' lightly dusted with the conidia of *Cunninghamella elegans* Lendner.

At the same time 500 g of each soil sample was analysed for the presence of various sporostatic factors known to occur in laboratory cultures of fungi [4 14]. The soil was first shaken with 250 ml of distilled water, filtered using Whatman no. 3 filter paper, and then centrifuged at 3,000 *g* for 10 min. The supernatant was examined directly by gas-liquid chromatography as described previously [14]. The combined residue from filtration and centrifugation was extracted five times with 1 l of petroleum ether (40°/60°) which was subsequently reduced to dryness, methylated, and analysed by gas-liquid chromatography as described elsewhere [4].

Results and discussion

The following fungi were isolated from the soil: *Rhizopus arrhizus* Fischer, *Rhizoctonia solani* Kuhn, *Cladosporium herbarum* (Pers.) Link ex Fr., *Absidia spinosa* Lendner, *Chaetomium globosum* Kunze ex Fr., *Penicillium claviforme* Bain, *P. frequentans* Westl., *P. brevicompactum* Dierckx, *Aspergillus fumigatus* Fres., *A. flavus* Link ex Fr., *Fusarium graminearum* Schwabe, *F. solani* (Mart.) Sacc., *Trichoderma viride* Pers. ex Fr., *Stemphylium macrosporoideum* (Berkeley & Broome) Sacc., *Alternaria humicola* Oudem., *Aureobasidium pullulans* (de Bary) Amand. When these were inoculated into sterilized soil samples, acetaldehyde, n-propanol and nonanoic acid were detected after 21 days incubation, and the germination of test spores of *C. elegans* was significantly retarded in these soils compared with that recorded in the sterile control sample (see Table 1).

TABLE 1

Sample	% germination at:				Concentration (ppm v/v) of:		
	3 h	24 h	48 h	72 h	Acetaldehyde	n–Propanol	Nonanoic Acid
Sterile soil	100 (96, 100)				0	0	0
Unleached inoculated soil	0 (0, 4)	10 (4, 19)	64 (54, 73)	97 (92, 100)	5.2	3.2	0.9
Leached inoculated soil	0 (0, 4)	6 (2, 14)	70 (60, 79)	100 (96, 100)	5.0	3.4	1.0

Figures in brackets represent 95% confidence limits. Error in determining concentrations = ± 5%.

Thus, the inhibition in these soils is associated with fungal metabolites which have been shown to be inhibitory to spore germination when assayed individually and to contribute to sporostasis in pure cultures of fungi [4 14]. Moreover, when acetaldehyde, n–propanol and nonanoic acid were added to the sterile sample at the levels recorded in the inoculated soils, the level of inhibition of spore germination which resulted was not significantly different from that in each of the inoculated soils (see Table 2).

TABLE 2

Sample	% germination of spores of *C. elegans* at:			
	3 h	24 h	48 h	72 h
Sterile Soil	100 (96, 100)			
Sterile Soil + acetaldehyde, n–propanol, potassium nonanoate	0 (0, 4)	8 (2, 16)	73 (63, 82)	98 (93, 100)

Figures in brackets represent 95% confidence limits.

This suggests that acetaldehyde, n–propanol and nonanoic acid could, under some conditions, function as sporostatic factors in soil. Possible mechanisms for the production of nonanoic acid in soil have been cited in an earlier publication [4] and acetaldehyde has previously been reported in soil [16].

Lingappa and Lockwood [7] have argued that sporostatic factors are formed in soil in response to nutrients leached from fungal spores or otherwise added to the soil. The present work indicates that nutrients liberated during

the sterilization of soil need not subsequently result in an increased production of sporostatic factors. Furthermore, even the low level of nutrients prevailing in a leached soil is adequate to permit the production of sporostatic factors.

Each of the factors identified here has also been found to be inhibitory to hyphal growth of the fungi listed above, although the concentrations required for significant effects were often relatively high. However, a solution of the three factors at the concentrations assayed in the unleached inoculated soil (see Table 1) caused a significant decrease in the hyphal growth rate of all of the species described. Thus, these sporostatic factors could contribute to the more general phenomenon of mycostasis in this soil. It is not suggested that these factors alone will explain mycostasis, or even sporostasis in soil. Previous work has emphasized the importance of a vacuolation factor in sporostasis in laboratory cultures [16], and since a vacuolation factor is widely distributed in soils [13] it seems likely that it could contribute to sporostasis there. Other factors, as yet unknown, may also prove to be important. However, in view of the widespread and general occurrence of simple fungal metabolites such as acetaldehyde, it seems reasonable that consideration should be given to such compounds as causal agents in soil mycostasis.

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